Actin cytoskeleton: **The Arp2/3 complex gets to the point**

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Actin filament polymerization results primarily from the addition of monomers to pre-existing filaments. Recent studies have revealed that the Arp2/3 protein complex, which includes two actin-related proteins, can nucleate new actin filaments, and this capacity can be enhanced by ActA, a protein used by *Listeria* to polymerize actin.

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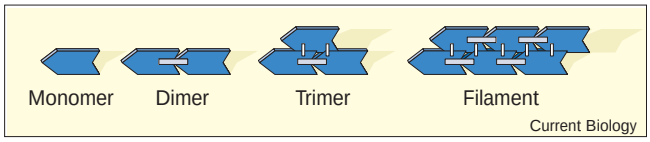
Cell locomotion depends on the polymerization of actin monomers to form organized filaments. Each filament has two distinct types of end, a pointed and a barbed end, named according to the pattern of arrowheads seen when myosin binds to the filament. Agonists that increase cell locomotion usually stimulate polymerization, and this occurs at the actin filament's barbed, or more rapidly growing, end. In a quiescent cell, barbed ends are mostly unavailable for further polymerization because they are 'capped' by actin-filament-capping proteins, such as capZ, which are present at high concentrations. Locomotion-promoting agonists are thought to create free barbed ends within the cell by uncapping and cutting existing actin filaments.

Now, Mullins *et al.* [1] have shown that the Arp2/3 complex of actin-associated proteins can create new barbed ends by nucleating new filaments from actin monomers. This nucleating activity of the Arp2/3 complex is quite low, but Welch *et al.* [2] report that it can be dramatically amplified by a protein, ActA, which is made by the intracellular parasite *Listeria monocytogenes*. The ActA protein has already attracted a great deal of interest among those studying the actin cytoskeleton because it is required for the formation of an actin-filament-rich comet-like tail behind each *Listeria* cell once the bacterium has penetrated into eukaryotic cytoplasm [3]. Interestingly, as well as nucleating new filaments, the Arp2/3 complex also caps the actin filament's pointed end, thus potentially inhibiting depolymerization. To appreciate the significance of these discoveries requires some background understanding of the nucleation of actin filaments and of the separate roles of the actin filament's barbed and pointed ends.

Figure 1



Filament formation from G actin monomers by nucleation. The actin dimer dissociates rapidly because the monomers are coupled only weakly, by one actin–actin bond. The trimer is more stable because the terminal actins are bound both along and across the filament axis. This simple model omits the effects of ATP hydrolysis.

Actin-filament nucleation

Addition of globular actin monomers (G actin) to an actin filament (F actin) during elongation, or loss of G actin during shortening, occurs at the filament ends. G actin can also form a new filament, by nucleation, but this process is slow. The rate-limiting step in nucleation is the assembly of actin monomers into a trimer (or tetramer). The first two monomers, coupled by a single actin–actin bond, dissociate rapidly, and only when a third monomer is added does the oligomer become stable. The trimer is more stable because the actin molecule at each end of the 'mini-filament' is coupled by two actin–actin bonds, one along and one across the axis of the filament (Figure 1). Thus, polymerization follows a sigmoid time course characterized by a lag period (nucleation) that can last for minutes.

In a test tube, purified monomers can be added to, or lost from, either end of the filament, but the rates of addition and loss are faster at the barbed end. Furthermore, when ATP is present, the barbed end has a somewhat higher affinity for G actin than does the pointed end. This difference results, at steady state, in 'treadmilling', the slow addition of monomers to the barbed end balanced by loss from the pointed end. In a cell, by contrast, accessory proteins greatly amplify the different kinetics and affinities of the two ends [4]. For example, profilin's binding of an actin monomer prevents addition of that monomer to the pointed end but not to the barbed end [5]. Profilin also facilitates exchange of ATP for ADP on G actin; this increases the amount of ATP-bound G actin, the form that adds to the barbed end. A second family of accessory proteins, the cofilin/actin-depolymerizing factor (ADF) family, enhances depolymerization at the pointed end [6]. The combined actions of profilin and cofilin convert the slow treadmilling of a pure actin filament to a rapid flux of monomers through the filament. In some cells, a third accessory protein, such as thymosin β4, provides a reservoir of G actin that allows a large change in the total amount of F actin to follow a small change in a filament's affinity for G actin [7].

Such a small change in affinity occurs when free barbed ends become available. In quiescent cells, most barbed ends are blocked by capping proteins, which are present

in an excess relative to barbed ends [8]. Agents that increase free barbed ends thus increase polymerization. So, a key question regarding polymerization *in vivo* is, how are barbed ends created? Free barbed ends can arise by uncapping existing filaments, by cutting existing filaments, or by nucleation of new filaments. Factors that create barbed ends by uncapping and by cutting are known. For example, phosphatidylinositol phosphates (such as phosphatidylinositol bisphosphate, PIP_2) uncap barbed ends by stimulating the dissociation of capping proteins (such as gelsolin and capZ) [9]. Members of the cofilin family can cut filaments [10], and both of the new ends created by the cut remain free, so cofilin's cutting increases both pointed and barbed ends. Gelsolin also cuts filaments, but it remains attached to the new barbed end, so gelsolin increases only pointed ends.

Until now, no factor had been identified that creates barbed ends through enhanced nucleation. Any molecule that binds to (and stabilizes) two adjacent monomers in a filament could, in principle, enhance nucleation. But, actually, only factors that bind two monomers across the filament axis seem to act as nucleators. Such factors include the barbed-end-capping factors gelsolin and capZ, and also the widely used actin-disrupting drug cytochalasin [11–13]. Given that each of these factors remains attached to the barbed end of the newly nucleated filament, however, only the pointed end is available for elongation. Thus, unless these factors are subsequently removed, their nucleating activity does not create free barbed ends.

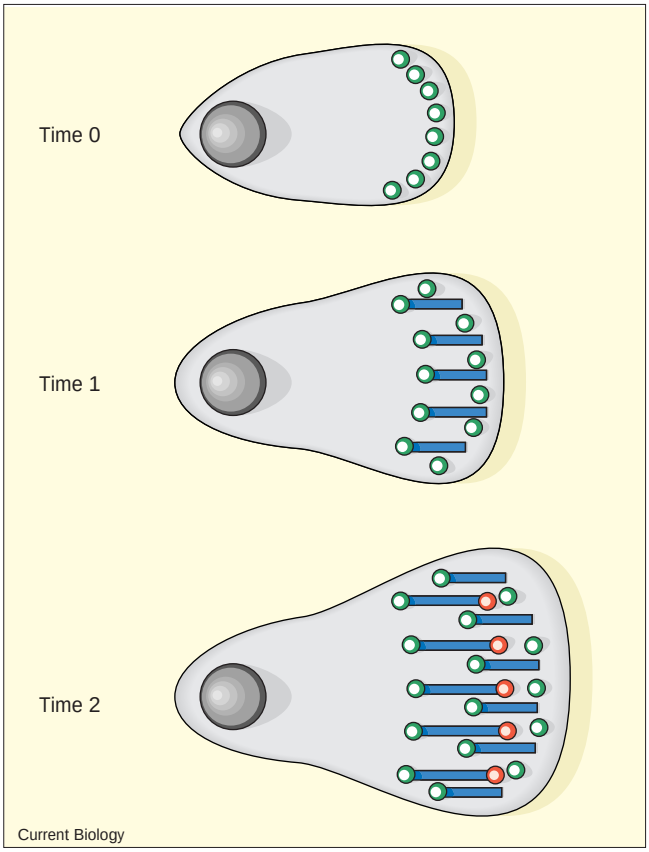
A new nucleator

Now a new nucleator has emerged: the Arp2/3 complex. This complex, which contains two actin-like proteins, Arp2 and Arp3, plus five additional proteins, was discovered to be a profilin-binding complex by Machesky and colleagues (for review, see [14]). The complex is found in eukaryotes from yeast to humans; in budding yeast, a mutation in either Arp2 or Arp3 is lethal [15,16]. The Arp2/3 complex is found near the front of lamellae in motile cells but is absent from stress fibers [17–19]. It also localizes to the F-actin tail induced by the *Listeria* parasite [20]. Thus, the Arp2/3 complex seemed to associate with rapidly turning over F actin but its function was unknown.

Last year, Welch and colleagues [20] showed that the Arp2/3 complex extracted from the platelet cytoskeleton stimulates clouds of actin filaments to accumulate around *Listeria*. Given that the Arp2/3 complex can cross-link F actin [21], it was unclear whether these clouds were due to the cross-linking of established actin filaments to *Listeria* or to local stimulation of polymerization. Now, Mullins and colleagues [1] demonstrate that the Arp2/3 complex can stimulate polymerization by enhancing nucleation. They show that the Arp2/3 complex, like previously described nucleators, is also an actin-filament capper. But unlike previously known nucleators, the Arp2/3 complex caps not the barbed end of the actin filament but rather its pointed end. Consequently, the new barbed end is free and might enhance polymerization *in vivo*. The Arp2/3 complex remains attached to the pointed end, blocking both polymerization and depolymerization [1]. At the front of a moving cell, the possibility that the Arp2/3 complex nucleates filaments and remains attached to their pointed ends could account for its presence through much of the lamella (Figure 2).

Nucleation by the Arp2/3 complex is modest: 2.3 μM Arp2/3 complex decreases the lag for polymerization (time to reach 10% of its final level) of 5 μM G actin about twofold (from 3 to 1.5 minutes). The reason, suggested by mathematical modeling, is that the Arp2/3 complex stabilizes actin dimers

Figure 2

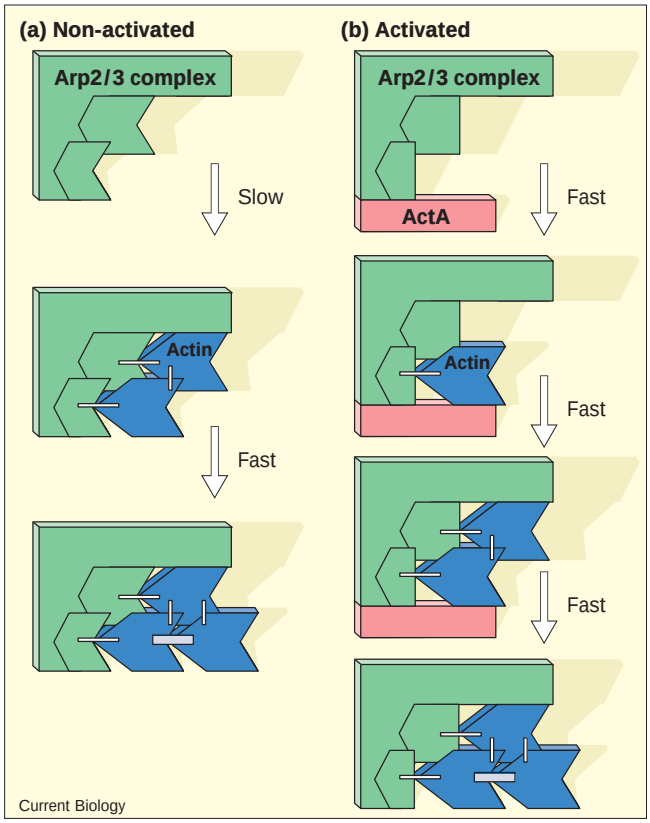


New filaments are nucleated at the front of a moving cell by Arp2/3 complex. As barbed ends extend forward, the Arp2/3 complex (green) attached to the pointed end becomes progressively further from the cell front where new filaments are nucleated. Thus, the presence of the Arp2/3 complex throughout much of the lamella is consistent with its hypothetical role in nucleating new filaments at the front. The distribution is equally consistent with filament formation by other means and the Arp2/3 complex capping the pointed ends; in either case, barbed ends eventually become capped (red dots).

whose concentration is very low (around 10^{-12} M *in vivo* if G actin is 0.5 μM) [1]. If micromolar Arp2/3 complex acted solely to stabilize dimers, even with a diffusion-limited 'on-rate', filament concentration could increase at only 10^{-11} M per second. Yet filament production stimulated by chemoattractants *in vivo* proceeds 10,000-fold faster (increasing at a rate of 10^{-7} M per second) [22,23]. To achieve that rate, activation of Arp2/3 must either expose a preformed nucleus in the complex, mimicking a barbed-end which can elongate by binding monomers, or must create binding sites for two or more monomers that would sterically resemble a barbed end (Figure 3).

Welch *et al.* [2] now report that ActA, the protein required by *Listeria* for actin polymerization, greatly enhances nucleation by the Arp2/3 complex. In the presence of 30 nM ActA, 30 nM Arp2/3 complex reduces the lag time to polymerize 2 μM actin by 20-fold (from about 5 minutes to about 15 seconds). Given that the protein concentrations in this experiment were low, the acceleration achieved by ActA may be sufficient to account for the rates *in vivo*. The activation of the Arp2/3 complex is mediated not by the polyproline-rich region of ActA but rather by the amino-terminal fragment, which is also required for *Listeria*-induced polymerization [3]. The amino-terminal fragment of ActA forms a dimer but it is not yet known if this is required for activity.

Figure 3



The nucleating activity of the Arp2/3 complex may be greatly enhanced by switching from a dependence on actin dimers to a dependence on actin monomers. (a) Nucleation by a non-activated Arp2/3 complex depends on its binding an actin dimer. This reaction would occur infrequently because dimers are present at very low concentration. (b) Activation may switch the mechanism to one dependent on binding actin monomers, which are present at a 10^5-times higher concentration than dimers. ActA could activate the Arp2/3 complex by contributing to the formation of a G-actin-binding site which could then enhance binding of a second G-actin monomer, appropriately spaced to produce a new filament. Apparently, ActA then dissociates from the nucleated filament.

Does the Arp2/3 complex nucleate actin *in vivo?*

Under what circumstances does the Arp2/3 complex nucleate new filaments? Given that ActA is absent from eukaryotic cells that are not infected with *Listeria*, there must be an intrinsic protein that can substitute, so a key step in future studies will be to identify this missing player. Furthermore, there may be additional factors that nucleate in a similar way to the Arp2/3 complex — by creating free barbed ends — and it will be important to search for them. Already, tropomodulin, the only other known pointed-end capping protein, has been observed to weakly nucleate actin filaments (A. Weber, personal communication).

Given the high affinity of the Arp2/3 complex for actin filament pointed ends (5–20 nM) and its cellular concentration (micromolar), Mullins *et al.* [1] suggest that all pointed ends in the cell are capped. This interesting proposal raises a problem. Given that depolymerization seems to occur at the pointed end, if all the pointed ends are capped, how could a filament depolymerize? Presumably by dissociation of the Arp2/3 complex, enhanced by other factors, or by filament cutting. The Arp2/3 complex on the pointed end could affect the timing and location of depolymerization. For example, in *Listeria* comet tails, the actin filament number decreases with time but the mean filament length stays constant [24]. Conceivably, dissociation of Arp2/3 is accelerated by cofilin, which might in turn allow depolymerization to occur so rapidly that filaments of intermediate length are not detected. In support of this idea, depolymerization of the *Listeria* tail is enhanced by addition of cofilin [25]. Clearly the new results implicating the Arp2/3 complex and an ActA-like activity in actin-filament nucleation have identified an important potential mechanism — nucleation — for regulating actin-filament growth, but there are many avenues which must be explored before we can say we understand how actin polymerization is regulated and how, in turn, it drives cell locomotion.

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